NITRITE

Jay B. Fox, Jr.*

*Agricultural Research Service

U.S. Department of Agriculture

Eastern Regional Research Center

Philadelphia, Pennsylvania 19118

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Over the past 14 years, efforts have been made to eliminate or lower the amount of nitrite in cured meats, the efforts resulting from the discovery that nitrite can form nitrosamines from endogenous food components. Most of the research during this period has been on nitrosamine formation, but research on the fate and function of nitrite in cured meats has not been entirely neglected. Nitrite in cured meats is a versatile compound. It colors and flavors, reduces some bacterial growth—importantly, that of Clostridium botulinum—prevents rancidity and, probably, improves texture. Some details on all of these functions are known, but there are still many mysteries. The mechanism of nitrite inhibition of clostridial spore outgrowth is not known. While some alkylnitrites and nitrates have been isolated recently from cured pork (Mottram, 1984), their presence still does not help explain nitrite's role in flavor production or rancidity prevention. The mechanism of color formation from nitrite and heme pigments is well defined, but the mechanism of color stabilization is not fully understood. Some of the nitrite added to meat during curing is either not readily determinable or accountable; why is not known. Let us examine some of the recent research on these subjects.

Chemistry

In all that follows, it must be remembered that the active form of nitrite is nitrous acid, the protonated form of the molecule (Figure 1). The transition point for this conversion is pH 3.4, the negative log of the

acid dissociation constant. This is well below the pH of cured meats (5.5 to 6.5) which means that the concentration of nitrous acid in cured meats is very low, 1.0 to 0.1% of the added nitrite, respectively. Nitrous acid is still not the active or nitrosating species but, by a bimolecular dismutation, forms nitrogen trioxide (Figure 1). The concentration of the nitrosating species is thus much lower than the nitrous acid concentration, which has led many investigators to use either high concentrations of nitrite or low pH, usually pH 3.0, to increase the acid concentration. Neither practice is necessarily relevant to nitrite reactivity in cured meats.

The reactions of nitrous acid are not the same at high concentrations as at low concentrations. Referring to Table 1, let us assume that at a given concentration of nitrite the reaction rates, column b, are equal for the three indicated reactions, column a. If the nitrite concentration is increased tenfold, column c, the rates of the three reactions are increased tenfold, hundredfold, and thousandfold, respectively. Obviously, the ratios of the reactants, intermediates, and products are going to be different at the two different concentrations. Extrapolation of data to lower nitrite concentrations is tenuous since in a highly complex system such as meat, the many reactants have differing reactivities with the varying products of the initial reactants.

Extrapolation of data from lower to higher pH values is tenuous since the reactivities of the many nitrite reactants available in meat have differing pH dependencies (Fox and Ackerman, 1968). Extrapolation from model systems to meat systems also may be misleading. For example, Fox et al. (1981) found no chloride/ascorbate interaction effect on residual nitrite in model systems, but a strong interaction in cured meat. The

bottom line in the study of any reaction still must be to show that the reaction actually occurs in cured meats at prevailing nitrite and hydrogen ion concentrations.

Nitrosation

Making a compound under one set of conditions and testing it under another is a technique that has been used to study transnitrosation. Transnitrosation is a special case of transfer reactions wherein a nitroso group is transferred from one compound to another.

$$RNO + R'H \qquad RH + R'NO$$

Nitrosocysteine is not produced in sufficient quantities at pH 5.5 to study its reactions, so Dennis et al. (1979) made the compound at pH 3.0, isolated it, and added it to model systems at pH 5.5. Nitrosation was observed, but the nitrosocysteine was unstable at pH 5.5, the amount of nitroso-product was less than that obtained from an equivalent amount of nitrite alone, and the results could have been accounted for completely in terms of free nitrite from the decomposition of the nitrosocysteine. Transnitrosation in a multiple substrate system could conceivably contribute to an increased amount of a nitroso compound, but it is unlikely. Starting with an activated species such as N_2O_3, the various nitrosatable species will react to produce a mix of products whose composition is dependent on the relative concentrations, activation and product energy levels of the reactant, and the temperature, pH, etc., of the system. In such a system, transnitrosation can play only a very minor role in the final equilibrium, since the latter is determined principally by the initial nitrosation reaction. Transnitrosation does not occur of course if the proposed nitroso-compound decomposes to yield the

original nitrosating species. I know of no study where any nitroso-compound formed *in situ* in meat accelerated formation or increased the amount of another nitroso-derivative.

"BCRT" Nitrite

When determining nitrite in meat there is some nitrite that is either slow to react or released only under special conditions. In the literature this nitrite is usually referred to as "bound" nitrite, but the term is too narrow and, in most contexts is used to refer to nitrite that is presumably reacted to some functional group in meat components. To be more precise, the term "BCRT" nitrite—that is, bound, complexed, reacted, or trapped nitrite—is used. This is an expansion on the term "BCR" nitrite (Fiddler, Personal communication).

Bound Nitrite

Nitrite ionically bound to special functional groups in meat is the simplest form in which the reactivity of nitrite ion could be reduced. Specific ion binding is very common in large polymers and is the basis for ion exchange chromatography. Such binding may occur on ionic sites in proteins, nucleic acids, and other endogenous polymers. Although the concept has not been studied, it would be surprising indeed if it did not occur in meats.

Complexed Nitrite

The cured meat pigment is the only form of nitrite in meat that can be positively determined. It is not nitrite per se, but nitric oxide (NO) produced from nitrous acid by reduction and complexed to the iron in the heme of the meat pigments, myoglobin and hemoglobin. In the native state one molecule of NO is complexed to the heme, but when the protein is denatured

by heat, the heme is freed from the protein and complexes a second molecule of nitric oxide. These two forms of nitric oxide complexes are readily quantitated, both as the pigment and, since the nitric oxide is readily dissociated, as nitrite.

Reacted Nitrite

Nitrite reacted with meat components is the most studied form and, refers to nitroso-derivatives of functional groups in the meat. Table 2 lists some potential reactants in meat.

Although the amide group of the peptide bond is present in very large quantities, there is no evidence that the corresponding nitrosamides are formed, nor from the known reactivity of these groups, would any be expected.

Amino acids have three types of nitrite reactive groups, amino, aromatic nuclei, and sulfhydryl. Kurosky and Hofmann (1972) studied the nitrosation of the amino and aromatic groups of amino acids at various acid pH values. Using their equations, I have extrapolated their data to the pH value of meat to derive the following estimates of the concentrations of nitrosated products after 24 hours of reaction (Table 3). The extrapolation did not take into account competing nitrosation reactions, equilibria, decomposition, etc., and the values therefore may be considered maximal. The indicated levels are mostly indetectable and do not contribute to BCRT nitrite.

The formation of nitrosocysteine has been studied extensively. It is assumed to be formed as an intermediate in the reduction of nitrite to nitric oxide by cysteine (Kelley and Watts, 1957). Mirna and Hofmann (1969) observed a concomitant and stoichiometric loss of nitrite and sulfhydryl groups during meat curing. Mirna (1970) subsequently found that mercuric

ions gave higher nitrite concentrations than did zinc ions and attributed the difference to mercuric ion cleavage of nitrosothiols, as reported by Saville (1958). The interpretation is not that simple. Mirna also showed that increasing the temperature resulted in decreasing nitrite concentrations with mercuric ion and increasing nitrite concentrations with zinc ion, until at 100°C both ions yielded the same amount of measured nitrite. Saville demonstrated cleavage only in strong acid solution; he did not study weaker acid conditions. Nitrosocysteine is not stable at pH 5.5 (Dennis et al., 1979), and whether or not mercuric ion contributes anything to cleavage at this pH has not been established. Recently, Byler et al. (1983) reported the formation of nitrosocysteine in a model system on the basis of an increase in absorption at 340 nm. However, Ito et al. (1979) following the formation of nitrosotryptophan by its absorption band at 335 nm, observed no increase in absorption at this wavelength in meat and concluded that nitrosotryptophan was not formed. We must conclude that nitrosocysteine isn't formed in cured meats either since both compounds absorb in the same region. With regard to the formation of nitrosocysteine, Kubberod et al. (1974) concluded on the basis of their study of the reaction of nitrite with myosin that the formation of nitrosothiols in cured meats was negligible. In summary, based on studies to date, it appears that nitrite does not react to any appreciable extent with amino acid functional groups.

Walters et al. (1979) have proposed the formation of pseudonitrosites from the reaction of nitrous acid and unsaturated fats, but their presence in cured meats has not been shown. Recently, Zubillaga et al. (1984) found a compound with antioxidant properties formed from polar lipids by reaction with nitrite, but they have not yet identified it.

Reductants are the strongest electron-donors, and nitrogen trioxide is an electrophilic reagent, hence, the most reactive of endogenous meat compounds are the reducing substances. The product of nitrite reduction via nitrous acid is the formation of nitric oxide. The naturally occurring reductants or reductones in meat are fully capable of the reduction, as evidenced by the formation of cured meat color. Ascorbate is a stronger reductant than the endogenous reductants of meat. On the basis of various reported rate constants, I have arranged the more important nitrosatable substrates found in meat in descending order of reactivity in Table 4.

Not only are reductant reactions with nitrite the fastest of all nitrosation reactions, but frequently they are multiple step reactions. For example, through a process of double bond migration, the nitrite oxidation of ascorbic acid proceeds by a number of steps to completely decompose the molecule (Figure 2). Some 33 different products of the oxidation of ascorbate have been isolated, of which only half have been identified. It is probably reductant or reductone compounds derived from carbohydrates that are the nitrite-containing compounds (Miwa et al., 1977 and Sebranek et al., 1978) isolated by chromatography. Some of these compounds were identified as organic acids, and in all of them the only nitrogen came from nitrite, which would be expected in carbohydrate products. None have been identified.

Trapped Nitrite

That nitrite might be merely trapped in cured meats was suggested by a recent observation I made in this laboratory while studying the determination of nitrite in various meat products. Lebanon bologna is a hard, fermented beef sausage made in Lebanon County, Pennsylvania. More nitrite was determinable from this hard product by 5 seconds of blending than by 2 minutes of fine

chopping. The blending produced a fine suspension while the chopping left discrete particles. Corroborating this observation, Olsman and van Leeuwin (1977) blended their sample rather than ground it as one of the procedures used to improve on Mirna's (1970) method of extracting nitrite. The simplest explanation for these observations is that the nitrite was trapped in the dense particles.

In summary, the form of "BCRT" nitrite—partly unavailable nitrite—is still not firmly established. It could be all, some, or none of the forms discussed. In this regard, it might be noted that almost all the nitrite in cured meats can be extracted by repeated extractions Rougié et al. (1980), but the observation itself says nothing of the form of "BCRT" nitrite.

Nitrite-Chloride Interactions

Because of recent efforts to reduce sodium chloride in meats, the interaction between nitrite and chloride is becoming increasingly important. The two compounds will form nitrosyl chloride, NOCl, which is a much more powerful nitrosating reagent than the nitrogen trioxide formed from nitrite alone, but there are some important differences between the two compounds. For example, chloride ion accelerates cured meat color formation, but the pink pigment is not as stable as it is in chloride-free systems (Sebranek and Fox, 1984). Chloride does not always accelerate nitrosation reactions; it inhibits nitrosopyrrolidine formation in cured meat systems. Finally, the products of the reaction of nitrosyl chloride frequently are not the nitroso derivatives; with trimethylamine, the major product is the chloride (Smith and Loeppky, 1967). In view of the differences reported in the literature between the kinds and quantities of products derived from N_2O_3 as compared to those from NOCl nitrosation reactions, it is extremely likely that such differences must also occur in meat systems.

Bacterial Inhibition

One of the most important functions of nitrite in cured meats is protection against microbial spoilage, particularly Clostridium botulin spore outgrowth. Roberts and Ingram (1973) studied outgrowth and toxin production of C. botulinum and found a very strong nitrite/chloride interaction (Table 5). Nitrite or chloride alone at normal curing levels had no effect on spore outgrowth, but the two together allowed only 42% germination.

Their data also showed an interesting relationship between nitrite and chloride with respect to the no risk level of the two salts (Figure 3). Below and to the right of the curve is the no risk area. Right in the middle of the area, where there is a finite risk of clostridial growth, are the nitrite and chloride levels—indicated by the exclamation mark—normally used in commercial meat curing. Obviously there is something more involved than just the reactions involving chloride and nitrite. Lowering of either nitrite, chloride, or both may raise the risk level to an unacceptable level. The risk increases disproportionally as the concentration of either chloride or nitrite is lowered (Figure 4). As can be seen in the figure, the decrease in nitrosyl chloride is proportionally greater in going from 2% down to 1% NaCl than it is in going from 3% to 2%, that is, the risk becomes proportionally greater as the salt, or equally, nitrite is lowered, and more extensive testing will be required to ensure that reduced salt products are completely safe. After all, it takes only one case of botulinum poisoning from a cured meat product to wreak havoc on the packing industry.

Cured Color Stability

Another area in which nitrite research brings us closer to some answers is in color stability of cured meats. As Tarladgis (1962) originally proposed, and Lee and Cassens (1976) proved, the cured meat pigment is the dinitrosyl

hemochrome. During processing, as the color begins to form, the initial red pigments are the native protein compounds, nitrosyl myoglobin and nitrosyl hemoglobin. As the temperature increases to the range of 155 to 170°F, these pigments denature, causing the heme to become free and react further with nitric oxide. From a theoretical standpoint, the dinitrosyl hemochrome is more stable than either of the two native red pigments. However, it must be recognized that these are not stable pigments, but readily dissociate to form the original reactants. Dissociation is accelerated by light, but if air is excluded the pigment will reform. For this reason, as Ramsbottom et al. (1951) showed many years ago, covering cured meats with an oxygen -impermeable wrap prevents light discoloration. However, even the latter is not totally effective, and further study needs to be made of the formation of the complex and the factors affecting its stability.

Curing with Nitric Oxide

From time to time, the direct use of nitric oxide has been proposed for cured meat color formation. The idea was investigated about 25 years ago by Harper et al. (1962) at Swift and Company. They developed a system for nitric oxide curing of frankfurthers, but the system was difficult to operate. High amounts of nitric oxide in the presence of even very low partial pressures of air caused the emulsion to turn green; too little nitric oxide resulted in poor color development. Beyond technical problems, nitric oxide is an insidious poison; at levels too low to detect by odor, it causes major lung deterioration. Nitric oxide is much more expensive than nitrite. In all, the disadvantages of using nitric oxide far outweigh any technical advantages.

In conclusion, we still have much to learn about the mechanisms whereby nitrite imparts color, flavor, protection and stability to cured meat products. Nitrite is a remarkably versatile compound and, to date, no compound has been

found that satisfactorily replaces nitrite for even one of its functions. It seems extremely unlikely that any one, or even any two, compound(s) will be found to replace it in all of its functions. Barring some extraordinary breakthrough, nitrite will continue to be used in curing meats.

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Table 1. Dependence of relative reaction rates on nitrous acid concentration.

Reaction (a)	Relative Rates $[HNO_2]$ (b)	$10[HNO_2]$ (c)
1. $HNO_2 + Cl \longrightarrow NOCl$	1	10
2. $2\ HNO_2 \longrightarrow N_2O_3$	1	100
3. $3\ HNO_2 \longrightarrow 2\ NO + HNO_3$	1	1000

Table 2. Potential nitrite reactants in meat, nitrite concentration = 2 mM.

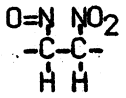
Reactant	mM	Products
Proteins		
Peptide	1500	Nitrosamides
Amino acids		
Cysteine	20	Nitrosothiol, RSNO
∝-amine	5	Nitrosamine, RNHNO, deamination
∈-amine	100	" " "
Aromatic	40	C-nitroso compounds
Hemes	0.1	Nitrosyl complexes, cured meat color
Fats (16% level)		
Unsaturated	500	Pseudonitrosites 
Polar lipids	?	Nitrosamines, antioxidants
Carbohydrates		
Reductones	100+	NO, nitric oxide
Coenzymes		
NADH	1	NO
Co A	0.03	"
Flavins	0.002	"
Added		
Ascorbate or Erythorbate	2	NO

Table 3. Nitrosation of amino acid functional groups at pH 5.5.

Reactive group	Amount after 24 hrs.
α-amino	7×10^{-7} M
ϵ-amino (lysine)	5×10^{-6} M
Indole (tryptophane)	2×10^{-5} M
Phenol (tyrosine)	10^{-5} M
Imidazole (histidine)	0
Pyrrolidine (proline)	none available

Table 4. The relative reactivity of various nitrite reactants with the nitrous acid/cysteine reaction rate value given the value of one. Compounds arranged in order of descending reactivity.

Compound or group.	Source	Reactivity
Ascorbate	Added	10
NADH	Coenzyme	2
-SH (sulfhydryl)	Cysteine	1
Indole	Tryptophane	0.02
Phenol	Tyrosine	0.002
ϵ-amino	Lysine	0.000002

Table 5. Percent outgrowth of Cl. botulinum spores as a function of nitrite and chloride concentrations.

	ppm $NaNO_2$	
%NaCl	0	100
0	100	100
2.5	100	42

Figure 1. Nitrous acid reactions.

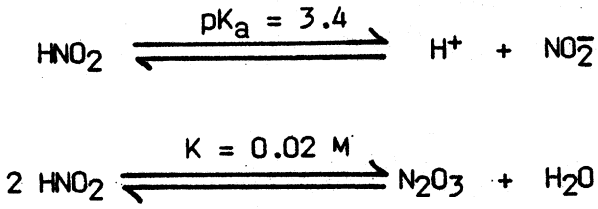


Figure 2. Nitrite oxidation of ascorbic acid.

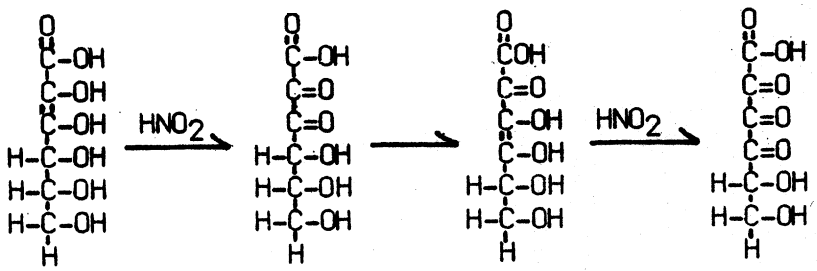
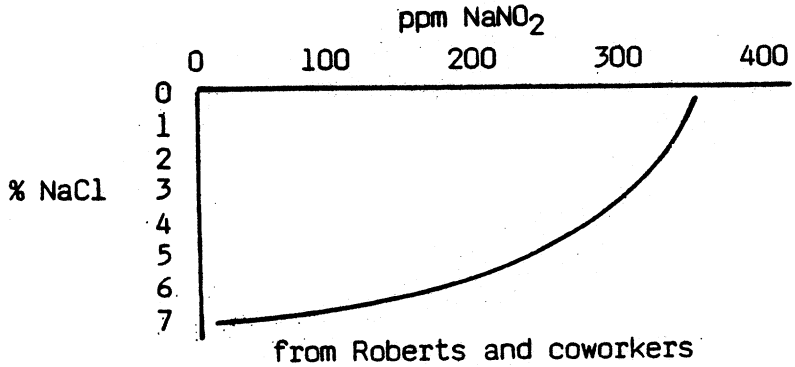


Figure 3. Assessment of risk of botulinal toxin production as a function of nitrite and chloride concentration.



from Roberts and coworkers

Figure 4. Amount of nitrosyl chloride produced as a function of varying salt concentration.